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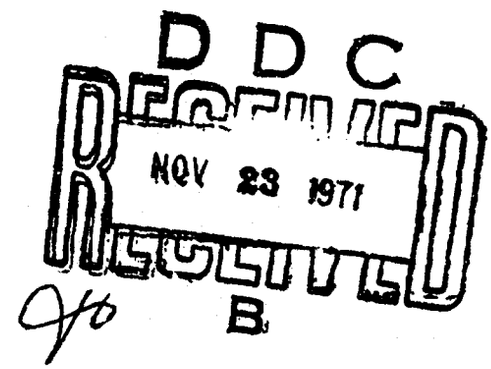

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Factors Determining the Level of Virus Production

Report: Effect of the Conditions of Cultivation on the Production of Several RNA-containing Viruses

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The effect of concentration of primarily trypsinized chick embryo fibroblasts on the level of the yield of Venezuelan equine encephaloymyelitis (VEE) virus per one cell under different conditions of cultivation--stationary monolayer, suspension, roller cultivation--was studied. Even though the greatest virus yield per cell is observed in monolayer stationary cultures, the maximum amount of cells counted per ml of growth medium without any significant reduction of virus yield per 1 cell is achieved in suspension cultures. In consecutive statistical comparison of the results of VEE virus cultivation, it was found that titers of the infectious activity in roller cultures were significant higher than in stationary monolayer and suspension cultures. Investigation of the regularities of VEE virus increase in primary and continuous cells in different cultural systems has been useful for production under these conditions of some other RNA-containing viruses.

In a previous report, the effects of the plurality of infection on the production of the VEE virus is different cultural systems was studied. Primarily only the trypsinized chick embryo fibroblasts were used in the capacity of model cells. Presently, the effect of the concentration of cells on the production of virus calculated on 1 ml of growth medium was investigated. The roller, suspension, and monolayer stationary of trypsinized and interwoven cells were primarily studied. Besides the Venezuelan equine encephalcymyelitis (VEE), other RNA-containing viruses were tested: a representative type of the A arbo-viruses----the Sindbis virus and mixo-virus of vesicular stomatitis virus(VSV).

Materials and Techniques

The described strain of VEE virus in the preceding report was used. The Sindbis virus was received from Granoff (Memphis, U.S.A.). The vesicular stomatitis virus, strain was received from the Museum of Virulent Strains of the D.I. Ivanovsky Institute of Virulogy, Academy of Medical Sciences, U.S.S.R. From the moment of delivery, the viruses were kept with serial passages in chick embryonic firoblasts (CEF). The viruses titrated according to the modified patch method with agar(14).

The trypsinized CEF were primarily prepared by the usual method(1). The interwoven cells of mice fibroblasts of L line(6) were grown in apparatus suspension cultivation. The interwoven cells of VERO line,----green marmoset kidneys, PS--pig kidneys(8), VNA-21----young Syrian hamster kidneys(11) breede in standard single liter mats in the form of one layer cultures. Cells were taken from the glass, with a mixture of the same volumes, warmed to 37 degrees 0.25% solution of trypsin and .02% solution of versin. Under virus cultivation in the capacity of growth medium and accumulation medium, the interwoven lines were exchanged for L cells and PS medium No. 199 with 10% normal heated beef serun, but for VERO and VNA-21 cells----the egla medium with 10% serum of calves. Antibiotics were added at the rate of 100 ED per 1 ml.

Results

Effect of the amount of cells calculated at 1 ml of accumulation medium on production of VEE virus.

The results, shown on the graph, are of the determining of average production of VEE virus calculated at one cell, obtained in a series of experiments characterizing the dependence of VEE virus production on the means of cultivation and density of cellular population (for suspension cultures) or the amount of cells on 1 ml of accumulation medium (for one layer stationary and roller cultures). Maximum virus production on one cell CEF in suspension was noted for density population $2.5\text{-}3\cdot10^6$ cells/ml and 1200 BOE/cells was

not exceeded. Optimum concentration of cells in roller cultures calculated at 1 ml of accumulation medium was 5-6•10⁶ cells/ml, but virus yield up to to 2500 BOE/cell was found in single layer stationary cultures of CEF cells in the quantity of 1 ml of accumulation medium equally with 1.5-2 mln.

Effect of manner of cultivation: In table 1 are represented the results of parallel VEE virus cultivation in suspension, stationary single layer, and roller cultures of CEF cells. Highest titers of infectious activity were determined under roller cultivation. Characteristically, here the effect of difference of virus titers in dependence from plurality of infection is strongly pronounced. Also systemitized in table 1 are results of VEE virus cultivation in different conditions on L cells and several interwoven lines of kidney cells ----PS, VERO, VNA-21. Roller cell culture turned out to be most productive. The change of the effect of plurality of infection in stationary single layer cultures in its dependence from the cell origin is to be noted.

In tables 2 and 3 are represented facts characterizing multiplication in different cultural systems of primarily — interwoven SV and VSV cells. Magnitude of maximum virus titers changes insignificantly under use of studied methods of cultivation. Effect of plurality of infection in these situations vaguely became apparent. Essential increase of infectious titers of SV virus when multiplying on interwoven VERO cells is noticeable in the event of use of roller culture in comparison with the stationary monolayer.

In a special series of experiments, possibility of equivalent application of the single layer method and suspended cultures for accumulation of several other representatives of RNA-containing viruses; Semliki timber virus, diseases of New Castle virus and A tyoe grippe viruses (strain WSN).

Roller and suspension cultures gave high concentration of viable cells on 1 ml of culture medium and therefore, in optimum conditions allow a high accumulation of hemagglutinin and interfon. So, in the multiplying of VEE

and SV viruses on CEF cells in suspension and on titer roller of hemagglutinin 2000-4000 hemagglutinin-producing units were achieved in one ml, but the activity of induced interfon with them, turned out to be, as a rule, near 1000 IE_{50}/ml and more. Detailed results of the study of higher accumulation of induced interfon with designated viruses in different culture systems were stated in previous communication.

Discussion

Cultivation manner and cell density--extremely vital factors which determine not only smoothness and outcome of infectious process in the interaction of viruses with cells, but also magnitude of average production of a virus on one cell. Maximum yield of a virus on a cell under use of single layer stationary cultures was reported.

Somewhat lesser production of a virus on one cell in roller cultures in comparison with one layer stationary is stated, obviously that in conditions of periodic contact of cells with nutrient medium in revolving bottles, the intensity of material change in them is lowered, but possibility of readsorption again of the produced virus grows. For the only, perhaps, exception concerning the fact of better virus production is of the tick encephalitis in suspension(12), lower production of virus on a cell in suspension is observed for versatile viruses with many researchers. In our experiments, virus production on one cell in suspension was continually lower, but in sufficient measure that production compensated with an increase in cell concentration. That question was more specifically discussed(among us) in another work(4).

Expediency of applied use of roller cultures are confirmed with an experiment of sucessful application of this method for obtaining anti-toxin vaccines (9, 10, Ubertini et al,1962). In relation of series of viruses, it was established that cultivation in revolving receptacles regularly encouraged virus suspension with sufficiently high titers(13, 2, 6, 5).

In statistical treatment of results, given in table 1 are veritable dif-

ferences between rows of indices in strength of the limited number of the given observations. Subsequently, statistical comparison of a big figure of experimental facts, received in different series of experiments according to virus multiplication in one layered stationary, roller, and suspension cultures, allow two conclusions: 1). the Fisher-Student index in comparison of roller and stationary cultures indicates reliability of differences and confirms the fact of higher virus yields in revolving containers, virus accumulation level in suspension and stationary single layer cultures are approximately similar; 2). with dispersing analysis, it seems to turn out well that among other factors determining magnitude of maximum virus titers(in the present case, roller in comparison with stationary), the role of the kind of cultivation is about 8%.

Comparison of different virus cultivation methods allow one important practical conclusion to be made: if single layer cultivation creates conditions for achieving high virus yields, then roller and suspension cultivation are more economical and make possible the obtaining of a virus in the shortest time.

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Non-Reparation of Damages Induced with 1 m of Hydroxylamine at Phage Lambda under its Passivation into E Coli HCR$^+$ and Coli HCR$^-$ Cells

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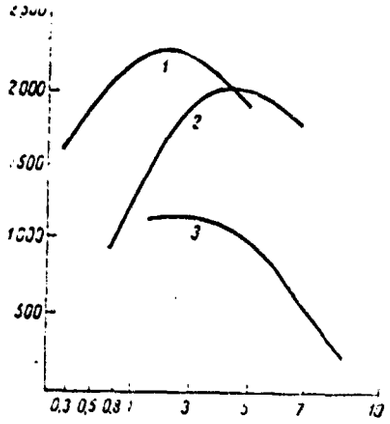
It is shown that E coli K12 hcr$^+$ and E. coli K12 hcr$^-$ do not differ accoriding to the ability to multiply of bacterophage lambda processed preliminary with a 1 m solution of hydrochloric hydroxylamine. Hydroxylamine interacting with cytosine residues in the DNA mosecule, converts them to oximes of cytosine. Findings lead to conclusion that enzyme system of dark reparation does not recognize changes of the secondary structure of DNA.

The problem of repair of genetic damages, induced by different agents of a radiant or chenical nature, acquired special significance in connection with the discovery of the possibility of the role of reparative system enzymes in such important life processes like recombination(7), replication(4,8) and mutagenesis(1, 3,5,). The question remains unsolved as to what kinds of DNA damages are known by the reparative systems. The opinion is expressed that certain kinds of chenical DNA disruptions do not undertake the repairs, rather the damages change secondary configuration of the DNA molecule(6).

Analysis of pepper mutagens whose damages in DNA undergo repair shows

that they all definitely can distort the regularity of double spirals of DNA, for mitomitsens, nitrous and sulfuric yperite, nitric acid can along with other effect suture DNA threads. Alkylating single function agents(methyl methane sulfate and ethyl methane sulfate) also disturb DNA secondary structure because of alkylation at N_7 at of guanine(see 3). If this is so, then it follows that under treatment with mutagens which will not significantly distort Watson-Crick DNA structure, damages necessitated by them will not by known by enzymes of the reparative system and repair will not take place.

It is known that hydroxylamine interacting with cytosines in DNA-structure and transforming cytosine residues into oximes, can turn out to be a minor influences on secondary DNA structure. In connection with what is expected, repair of damages from the indicated agent will not follow. In present experiments, this was confirmed by the influence of hydroxylanine in vitro at phage lambda and subsequently its cultivation in E coli hcr$^+$ and E coli hcr$^-$ cells.



VEE virus production on one CEF cell in different conditions of cultivation

On ordinate axis - virus production (in BOE/cell)
On abscissa axis - amount of cells ($\cdot 10^{-6}$) on 1 ml of accumulation medium

1 - single layer, 2 - roller, 3 - suspension

Table 1 - Accumulation of VEE Virus in Primary and Interwoven Cells in Different conditions of Cultivation (in lg BOE/ml)

Клетки 1	Множественность инфекции (в БОЕ/клетка) 2	Монослой 5		Роллер 6		Взвесь 7	
		24 часа 3	48 часов 4	24 часа 3	48 часов 4	24 часа 3	48 часов 4
CEF	1—10	8.9	8.4	7.2	8.0	8.6	7.9
	10^{-1}—10^{-6}	9.5	9.0	9.05	9.2	9.0	8.3
L	1—10	8.3	8.4	—	—	8.1	8.2
	10^{-1}—10^{-6}	4.2	5.3	—	—	—	—
PS	1—10	7.0	8.8	8.6	8.6	7.7	8.1
	10^{-1}—10^{-6}	5.0	7.0	—	—	5.8	6.1
VERO	1—10	7.7	8.0	8.3	8.6	7.0	7.1
	10^{-1}—10^{-6}	7.1	8.0	—	—	—	—
VNA 21	1—10	7.3	7.0	8.4	9.0	7.3	7.1
	10^{-1}—10^{-6}	7.7	7.4	—	—	—	—

1= cells; 2= plurality of infection (inBOE/cell); 3=hour; 4= hr 5=single-layer 6= roller; 7= suspension

Table 2 - Accumulation of VSV virus in cultural conditions (in lg BOE/ml)

Cells / Множественность инфекции / 1	Монослой 2		Ролер 3		Взвесь 4	
	24 часа 5	48 часов 5	24 часа 5	48 часов 5	24 часа 5	48 часов 5
Primarily trypsinized CEF						
1—10	8,7	7,0	—	—	8,3	7,0
10^{-5}—10^{-6}	6,9	6,0	6,0	—	6,9	7,4
Interwoven line RS 1—10	6,7	6,0	—	—	7,2	5,5

1= Plurality of infection; 2= single-layer; 3=roller; 4=suspension
5= hours

Table 3 - Accumulation of sindbis virus in different cultural conditions (in lg BOE/ml)

Cells / Множественность инфекции / 1	Монослой 2		Ролер 3		Взвесь 4	
	24 часа 5	48 часов 5	24 часа 5	48 часов 5	24 часа 5	48 часов 5
Primarily trypsinized CEF						
1—10	9,1	7,3	9,1	8,1	9,1	—
10^{-5}—10^{-6}	9,2	9,1	8,7	8,2	9,3	8,5
Interwoven lines of VERO 1—10	6,2	7,8	7,0	8,9	—	—

1= Plurality of infection; 2= single layer; 3=roller; 4=suspension
5=hours.